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THE EFFECT OF SMALL HYDROSTATIC PRESSURE GRADIENTS ON THE RATE OF ACTIVE SODIUM TRANSPORT ACROSS ISOLATED LIVING FROG-SKIN MEMBRANES

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SUMMARY

1. The rate of active sodium transport across living isolated skins from *Rana temporaria* was measured when the skins were bulged inwards and outwards by small constant hydrostatic pressure gradients and by pushing them mechanically in the absence of such gradients. The effect of pressure gradients in the absence of bulging was also studied.

2. An apparatus was designed to circulate Ringer solution to each side of the skin at constant temperature, flow and pressure. The pressures were controlled to within 0·5 mm H_2O.

3. It was found that bulging the skins in the absence of hydrostatic pressure gradients had no effect on sodium transport but that pressure gradients of less than 5 mm H_2O had a marked effect, increasing transport when the pressure was higher on the outside of the skin, and decreasing it when the pressure was higher on the inside.

4. It is concluded that increasing surface area does not influence sodium transport, whereas small hydrostatic pressure gradients have a marked effect.

5. Possible causes for this phenomenon are discussed and its significance is considered with special reference to sodium reabsorption from the proximal tubule of the kidney.

INTRODUCTION

An investigation has been carried out to determine the influence of small hydrostatic pressure gradients on active sodium transport across isolated living frog-skins. The effect of small gradients has not previously been studied in amphibian membranes, but R. A. McCance & D. M. Nutbourne (unpublished work), have found that such gradients profoundly influence sodium transport across pig chorio-allantoic membranes.

In the kidney a relationship has been shown between the rate of sodium reabsorption from the proximal tubule and the size of the tubule. Gertz (1963) has suggested that it varies proportionally with the square of the radius, and Rector, Brunner & Seldin (1966) and Brunner, Rector & Seldin (1966) have concluded that in states of glomerulotubular balance, sodium reabsorption varies directly with tubular volume. It seems likely that tubules dilate and constrict because there is a change in hydrostatic pressure gradient across the tubular wall (Earley & Friedler, 1966; Martino & Earley, 1967). In the intact kidney it has not been possible to determine whether the rate of sodium reabsorption is influenced primarily by the hydrostatic pressure gradients across the tubular wall or by the changes in the wall which accompany dilatation or contraction (such as changes in the surface area of the tubular epithelium, movement of the entire tubular wall and changes in the position of the individual cells relative to each other). Therefore, the relative importance of these factors has been investigated using isolated living frog-skins.

The rates of active sodium transport were compared when frog-skins were bulged towards the inside and towards the outside by means of constant hydrostatic pressure gradients and also when they were bulged by pushing them mechanically in the absence of such gradients. The effect of hydrostatic pressure gradients in the absence of bulging was also studied. Thus, the effect of increasing surface area, together with membrane movement and changed relative positions of the membrane cells, was studied in the presence and absence of a hydrostatic pressure gradient, and the effect of a pressure gradient was studied in the absence of a change in surface area or membrane movement.

METHODS

Apparatus

The circulation of the frog Ringer solution

An apparatus (Fig. 1) was designed in which pressures could be independently controlled on each side of the membrane without changing the flow, temperature or composition of the Ringer solution. A constant flow of oxygenated solution was supplied by identical, unconnected circuits to each side of the membrane. Ringer solution was pumped by means of a Watson–Marlow M.H.R.E. Flow-Inducer from the reservoir, R_1, on the floor to the top 'feed' Perspex reservoirs, R_2 and R_3, about 250 cm above the floor. In R_2 and R_3, the solution was oxygenated by bubbling washed, humidified air through it. From the bottom of R_2 and R_3 there were two outlets: a Perspex tube, O, through which the solution flowed to the membrane cell, and a wide chimney, Q, which protruded a distance of 12 cm above the bottom of the cylinder and was connected by wide silicone-rubber tubing directly to the bottom reservoir, R_1. The level of the fluid was kept constant in R_2 and R_3 by ensuring that there was always an overflow through Q.

The solution passed from O through a Hone Needle-Valve, U, and a Rotameter Flowmeter, F, and temperature was adjusted as the fluid passed through a coil of glass or polyethylene tubing immersed, together with the cell, in a water-bath, W, kept at 20° C by a Circotherm

heater and circulator. The fluid entered the membrane chamber at A and emerged at B (Fig. 2); it then passed through capillary tubing to a glass drip-chamber, Z, open to the air, and from there fell to the common reservoir, R_1. Thus, once in each circuit, the solutions from the two sides of the membrane were mixed in R_1. Silicone-rubber tubing was used in the circuits.

Control of pressure and flow. In order to change the hydrostatic pressure in the cell without at the same time changing the flow, it had to be possible to alter the height of the top reservoirs above the cell without altering the vertical distance between these reservoirs and the drip-chambers. Therefore, in each circuit the top reservoir, needle-valve, flowmeter and drip-chamber were all clamped onto the same vertical metal rod. These rods were mounted so that each could be moved vertically independently of each other or both could be raised or lowered together as a single unit; thus, the heights of R_2 and R_3 above the

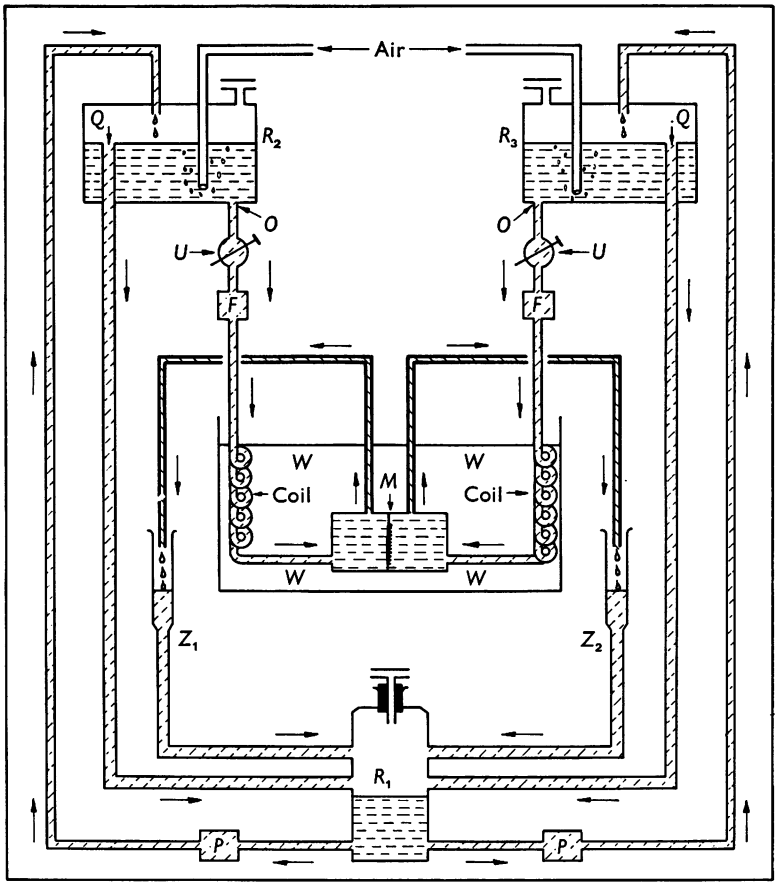

Fig. 1. A diagram of the circulation of Ringer solution. M is the frog-skin membrane; W, the water-bath at 20° C; R_1, the common reservoir; P, a pump; R_2 and R_3, the 'feed' reservoirs; Q, the overflow chimneys; O, the exits from R_2 and R_3 into the membrane cell circulations; U, the needle valves; F, the flowmeters and Z_1 and Z_2, drip chambers open to the air.

membrane chamber could be varied independently or simultaneously, while the distance between the reservoirs and their corresponding drip-chambers remained constant.

To keep the hydrostatic pressure constant in the membrane chambers, a constant flow rate was necessary, and a rate of 30 ml./min was chosen because it was found that the short-circuit current was independent of flow at rates above 20 ml./min. Flows were adjusted by needle-valves. The lengths and types of tubing were identical in the two circuits, and, to facilitate pressure control, wide-bore tubing was used on the inlet side of the membrane cell and small-bore, thick-walled tubing on the outlet side. To maintain constant pressures and flows, the apparatus was kept as clean as possible and there were no kinks in the tubing or air-bubbles in the circuits. Under these conditions, the pressures in the membrane chambers could be kept constant to within 0·5 mm H_2O pressure for several hours.

Flow was measured with glass Rotameter Flowmeters and hydrostatic pressures were measured on each side of the membrane by Statham Physiological Pressure Transducers P23BB, and recorded on a two-channel Cardiac Recorder Type 10C, using a film speed of 1 mm/sec; 1 cm deflexion on the scale represented 1·67 mm H_2O pressure; strain gauges were so positioned that the zero pressure was level with the centre of the membrane.

The Perspex membrane cell

By using a variety of interchangeable components, the cell could be used in several different types of experiment. The membrane was fixed vertically in a separate unit between the two chambers of the cell. Into each of the membrane chambers, i.e. on each side of the membrane, there were seven openings, *A*, *B*, *C*, *D*, *R*, *S* and *T* (Fig. 2). *A*, the inlet for Ringer solution, was at the top of the chamber and was directed so that the incoming solution impinged at the bottom edge of the membrane in the mid line. *B*, the outlet for the solution was in the mid line at the highest point in the chamber, near the membrane and as vertical as possible. *C* and *D* entered the chamber laterally, one on each side of the membrane; they were horizontal, level with the centre of the membrane and directed towards its centre. *C* was connected by a wide silicone–rubber tube to the equivalent opening in the other membrane chamber to form an optional pressure-equalizing system. *D* was connected to a strain-gauge which measured hydrostatic pressure in the cell. Perspex fittings were designed to form watertight connexions at *A*, *B*, *C* and *D*.

There were three openings, *R*, *S* and *T*, at the distal end of both chambers, each directed towards the centre of the membrane; *T* was horizontal, at right angles to the centre of the membrane, and through it were inserted the Perspex devices used to bulge the membrane; through *R* and *S* passed the KCl-agar leads used in the measurement of membrane potential and short-circuit current. A fitting was designed to screw into the cell at *R*, *S* and *T* and form a connexion which was watertight but did not compress the KCl-agar leads of the electrical circuits at *R* and *S*.

The membrane mounting unit consisted of two pairs of disks, an inner pair in which the membrane was mounted and an outer pair in which the inner pair and membrane were fixed. The periphery of the outer disks was gripped between the two halves of the cell as the main clamping-screws were tightened, and the inner disks had flanges which interdigitated with each other and with corresponding flanges in the outer disks. There were two kinds of outer disk, one with large holes round the perimeter to allow free communication between the chambers on either side of the membrane and the other with no holes so that the two chambers were isolated from each other. There were also two kinds of inner disk, one fixed the membrane firmly round the perimeter but allowed free movement of the centre of the membrane and the other type had grids across them made from thin, 0·02 in. (0·508 mm), Perspex sheets to limit bulging of the membrane. These grids lay one on each side of the membrane with their adjacent surfaces 0·1 in. (2·54 mm) apart. The diameter of the membrane was 1 in. (25·4 mm).

The fitting which was used to bulge the membranes mechanically was a Perspex umbrella-

shaped device, *E*, attached to a Perspex rod (Fig. 2). The 'umbrella' was made from 8 ribs of Perspex, joined in the mid line and curving outwards to join a circular Perspex ring. When *E* was pushed up to the membrane, the circular Perspex ring exactly fitted against the inner disks of the central membrane-fixing-unit so that the maximum bulge for the membrane was constant with the centre of the membrane displaced by 0·25 in. (6·35 mm). Whenever a membrane was bulged by a pressure gradient, the fitting *G* was used to measure the extent of the bulge. It consisted of a small Perspex block fixed to a rod. Mounted on *E* and *G* outside the cell were vertical Perspex pointers which moved along a scale fixed to the cell.

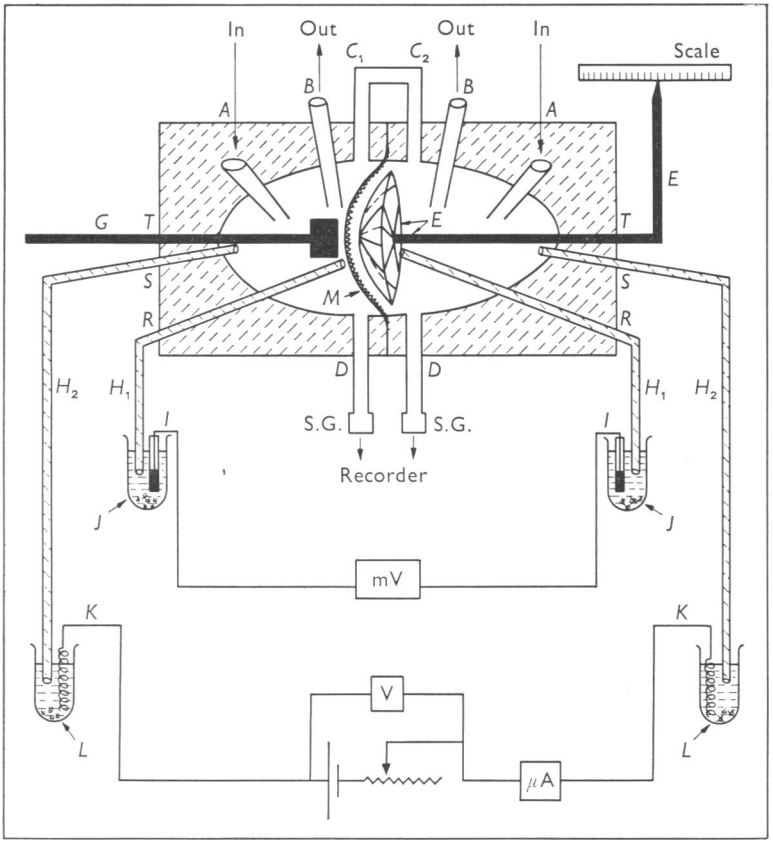

Fig. 2. A diagram of the membrane cell with its attachments and the electrical circuits. *M* is a frog-skin membrane. Into *A* is fixed the inlet and into *B* the outlet for Ringer solution; into C_1 and C_2 the optional pressure-equalizing tube and into *D* the strain gauges, S.G., measuring pressure. Into *R* and *S* pass KCl-agar leads for measuring membrane potential and short-circuit current; into *T* are fixed the Perspex devices *E* and *G*. *E* is the Perspex fitting for bulging the membrane and *G* is the device for marking the extent of membrane bulging; H_1 and H_2 are polythene leads containing KCl-agar; *J* is a tube containing saturated KCl; *I*, a calomel half-cell; *K*, a silver wire in *L*, a tube containing saturated AgCl in saturated KCl.

Composition of the frog Ringer solution

The solution contained 93·3 m-equiv/l. Na^+; 1 m-equiv/l. K^+; 1·3 m-equiv/l. Ca^{2+}; 93·3 m-equiv/l. Cl^-; 1·2 m-equiv/l. HCO_3^-; 0·3 m-equiv/l. $H_2PO_4^-$; 0·8 m-equiv/l. HPO_4^{2-} and 700 mg/l. dextrose; pH was adjusted to 7·4.

Electrical circuits used in the estimation of active Na^+ transport across frog-skins

Active sodium transport across the membrane was estimated by the short-circuit current method of Ussing (1949). Membrane potential was measured with a Vibron Electrometer 33 B2, and the backing-off circuit consisted of a 120 V multisocket dry battery, a voltmeter, a potentiometer, and a Precision Microammeter (Cambridge Instrument Co.) (Fig. 2).

The positioning of the KCl-agar leads within the membrane cell was critical. To give the most uniform electrical field over the membrane during the measurement of short-circuit current, the ends of the KCl-agar leads carrying the backing-off voltage were placed just inside the membrane chamber at *R*, *S* or *T*. The leads used in the measurement of membrane potential were always placed as close to the centre of the membrane as possible, but in experiments when the membrane was to be bulged they were placed so that bulging could take place without the skin touching them. The leads were positioned when the membrane was first mounted in the cell and were *never moved* throughout the experiment. Therefore, the electrical resistance of the Ringer solution between the leads remained constant and changes in the measured short-circuit current were proportional to changes in the absolute short-circuit current. Under these conditions it was found that the membrane potential and short-circuit current were identical when the two membrane chambers were isolated from each other and when they were connected through the holes in the outer disks of the membrane-holding unit.

To prevent leakages from the high-voltage backing-off circuit to the low-voltage circuit, all leads were screened and the voltage used to back-off membrane potential was kept as low as possible by making the KCl-agar leads used in the high-voltage circuit as short as possible and not compressing them in the cell fittings.

Techniques used in setting up an experiment

The membrane cell was assembled using the disks, Perspex devices and positioning of the KCl-agar leads appropriate to the experiment to be performed. The cell was joined to the circuits containing Ringer solution, tested for leaks and put into the water-bath. All bubbles were removed and flows were adjusted to 30 ml./min in each circuit. R_2 and Z_1 were adjusted to be at the same heights as R_3 and Z_2 respectively and the hydrostatic pressure in the cell was adjusted to be about 2–4 cm H_2O above atmospheric pressure so that, in the event of a leak, water from the water-bath would not enter the cell.

The frogs used were *Rana temporaria*, kept at room temperature on growing grass with access to a tank of deionized water. They were killed by decapitation and then pithed. The maximum area of abdominal skin was removed under Ringer solution, care being taken not to stretch the skin during the dissection. The smaller of the inner disks was fitted into the appropriate outer disk and the membrane was floated over it with the inside of the skin facing downwards. The larger of the inner disks was fitted over the membrane and pressed into the appropriate groove on the outer disk. Excess skin was cut off and the second outer disk was fitted into place. The cell was separated from the Ringer solution circuits and taken from the water-bath and, as quickly as possible, the membrane mounting unit was put between the halves of the cell and the clamping-screws were replaced; then the chambers were reconnected to the circuits and air bubbles were removed. As the chambers were filling, the membrane was observed continuously because the slightest inequality of filling or a small trapped air bubble quickly caused severe bulging and damage to the membrane. When the membrane had no tendency to bulge, the cell was replaced in the water-bath. C_1 and C_2

were connected by tubing so that there was no hydrostatic pressure gradient across the membrane.

Flows were maintained at 30 ml./min and the hydrostatic pressures in the chambers at 2–4 cm H_2O above atmospheric pressure. At intervals of 5 min, flows and pressures were measured and, if necessary, readjusted, and measurements were made of membrane potential and short-circuit current. When the short-circuit current reached a steady state, the skin was short-circuited permanently with zero potential difference across the membrane. It then remained steady or showed an even rate of fall for 12–40 hr.

Experimental procedures

The first control period of 1–3 hr started as soon as the short-circuit current had reached a steady state. About 30 min before the end of the first control period, the camera was switched on to record the hydrostatic pressures in the membrane chambers, so that small transient changes in pressure could be detected. Each experimental period lasted 1–3 hr and was followed by a second control period of 1–3 hr. Throughout this time, short-circuit current, flows and pressures were measured every 2–5 min. The experiments performed were:

(*a*) *The effect of changing hydrostatic pressures equally on both sides of frog-skins*

Outer disks containing holes and inner disks with grids were used and C_1 and C_2 were kept connected throughout the experiment. During the initial control period the pressures were kept either at 100 or at 1 cm H_2O. Then the pressures were either raised to 100 cm H_2O from 1 cm H_2O or were lowered from 100 cm to 1 cm H_2O simultaneously in both membrane chambers. After the experimental period, the pressures were simultaneously returned to their first value and the second control period followed.

(*b*) *The effect of bulging frog-skins by small constant hydrostatic pressure gradients*

Outer disks without holes and inner disks without grids were used and *G*-type fittings were placed on each side of the membrane, 0·25 in. (6·35 mm), from the mid line. At the end of the initial control period, the tubing connecting C_1 and C_2 was occluded and the pressures in the membrane chambers were adjusted until the frog-skin was bulged with its centre just touching *G*. The pressure gradients needed varied from 2 mm H_2O in a loosely mounted skin to 60 mm H_2O in a tightly mounted skin. The membrane was watched continuously and the bulge was kept constant throughout the experiment. Then the chambers were reconnected through C_1 and C_2 and the relative heights of R_2 and R_3 were readjusted so as to be the same as those in the first control period, and the second control period followed.

(*c*) *The effect of constant hydrostatic pressure gradients across frog-skins in the absence of bulging*

Outer disks without holes and inner disks with grids were used. The experimental procedure and precautions were exactly the same as in the preceding experiment (*b*) except that the grids prevented bulging of the membrane. The pressure gradient was adjusted so that the membrane did not bulge through the holes of the restraining grids. Pressures were watched continuously to ensure that they did not change.

(*d*) *The effect of bulging frog-skins by pushing them mechanically*

Outer disks with holes and inner disks without grids were used and the membrane chambers were kept connected through C_1 and C_2. After the first control period the 'umbrella'-shaped *E*-type device was gently advanced, pushing the membrane in front of it until the centre of the membrane had moved 0·25 in. (6·35 mm). After 1–2 hr, *E* was withdrawn so that the membrane returned to the mid line and the second control period followed.

(*e*) *To compare the effects of bulging a frog-skin to the same extent by pushing it mechanically and by applying a constant hydrostatic pressure gradient*

Outer disks without holes and inner disks without grids were used. A *G*-type device was fitted into one chamber and an *E*-type device into the other. The aim of this experiment was

to keep a constant bulge using first E and then a hydrostatic pressure gradient, the transition being effected without any movement of the membrane. Each stage lasted 1–3 hr.

Stage 1. After the first control period, C_1 and C_2 were kept connected and E was gently advanced until the centre of the membrane had moved 0·25 in. (6·35 mm). The position of G was adjusted so that it just touched the convex surface of the membrane. Short-circuit current, flows and pressures were measured for 1–3 hr.

Stage 2. The tubing connecting C_1 and C_2 was occluded and the pressures in the chambers were adjusted to keep the membrane just touching G. E was then withdrawn and the membrane was watched continuously to ensure that the bulge remained constant throughout the experiment.

Stage 3. E was again advanced to fit into the bulge of the membrane and then C_1 and C_2 were reconnected to equalize the pressures. Short-circuit current, flows and pressures were measured for 1–2 hr.

Stage 4. C_1 and C_2 were reconnected so that the membrane returned to the mid line and a second control period followed.

RESULTS

The effect of changing hydrostatic pressure equally and simultaneously on both sides of frog-skins in the absence of a pressure gradient across the skins (Fig. 3)

In all of the thirty-six experiments performed it was found that a change of pressure of 99 cm H_2O had no significant effect on the short-circuit current in the absence of a pressure gradient across the membrane.

The effect of bulging frog-skins inwards and outwards by means of small constant hydrostatic pressure gradients (Fig. 4)

172 frog-skins were studied. When the skins were bulged outwards, there was in all cases an immediate and sustained fall in current which continued for at least an hour before a new low stable level was attained. When skins were bulged continuously towards the inside by a constant hydrostatic pressure gradient, the short-circuit current rose, reaching a peak after about 30 min; in most skins the current then slowly fell to below control values, but in two experiments when the pressure gradients used were 2 and 6 mm H_2O (Fig. 4), the short-circuit current remained above the control value. The speed of response varied with the pressure gradient, the smaller the gradient, the greater the interval before the current reached its peak, i.e. at 60 mm H_2O the peak was reached after 20 min whereas at 2 mm H_2O it was not reached for an hour. In most skins the amount that the current rose was greater with low pressure gradients than with high gradients.

When pressures were equalized and the membrane returned to the mid line, whatever the direction of the previous bulge, the short-circuit current showed a similar response: the current rose for 30–60 min and then fell

slowly for 2–3 hr towards the value in the control period; this response was immediate after bulging outwards and delayed after bulging inwards.

After any change in pressure gradient, the short-circuit current did not reach a new stable state for 1–4 hr, and the higher the initial short-circuit current the greater was the response to the gradient. Although it was not

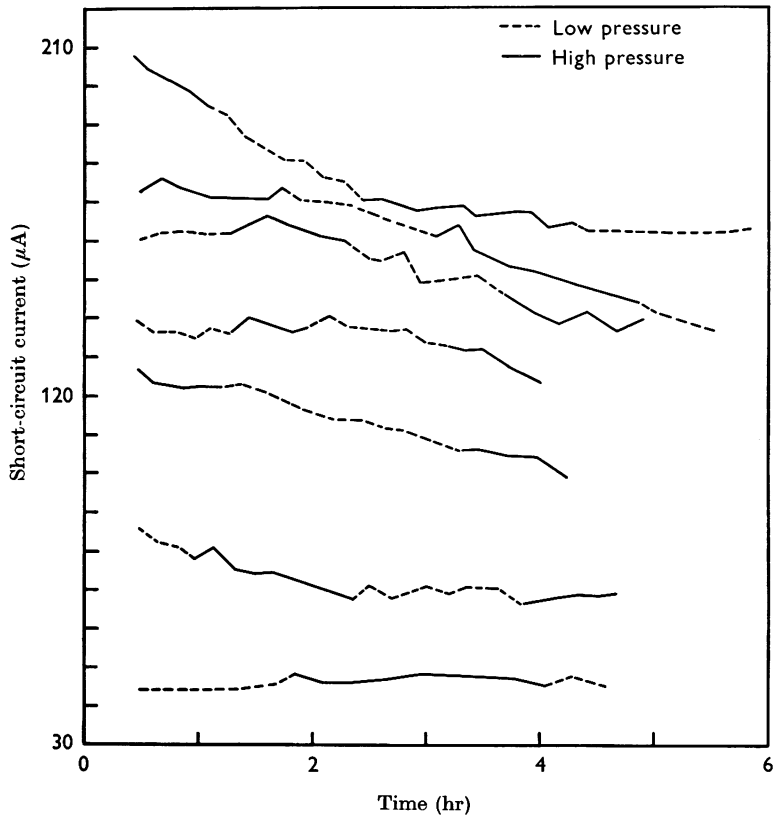

Fig. 3. The effect on the short-circuit current (μA) of changing hydrostatic pressure simultaneously on both sides of living frog-skin membranes, in the absence of any pressure gradient. Continuous lines represent the short-circuit current when hydrostatic pressures were 100 cm H_2O on both sides of the membrane and interrupted lines represent the current at pressures of 1 cm H_2O respectively.

found possible with the apparatus used to maintain a constant pressure gradient of less than 0·5 mm H_2O for several hours, it was frequently noticed that transient gradients of 0·1–0·5 mm H_2O had a pronounced effect on the short-circuit current, the direction of the changes being consistent with those found using larger gradients.

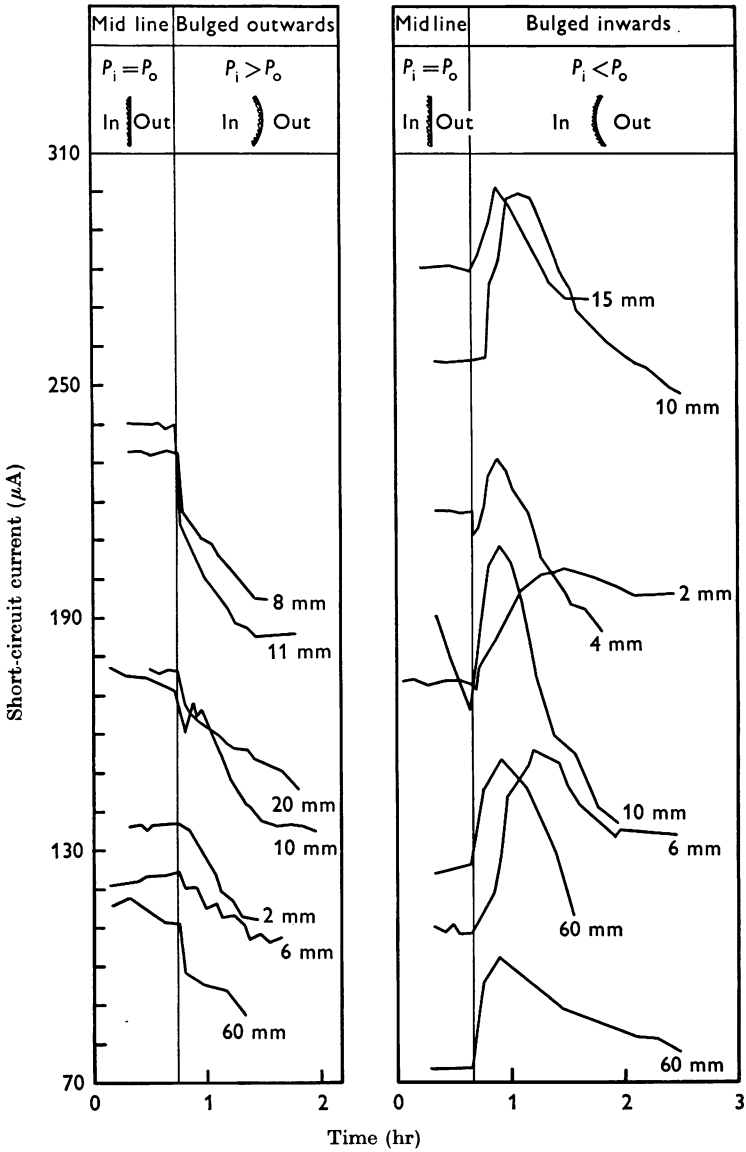

Fig. 4. The effects on the short-circuit current (μA) of bulging isolated living frog-skin membranes by means of small constant hydrostatic pressure gradients. In each case the centre of the membrane was moved 0·25 in. (6·35 mm), and the surface area was increased by 25%. The figures on the right of the lines show the pressure gradients in mm H_2O needed in each skin to produce the required bulge. P_i and P_o are pressures inside and outside the skin respectively.

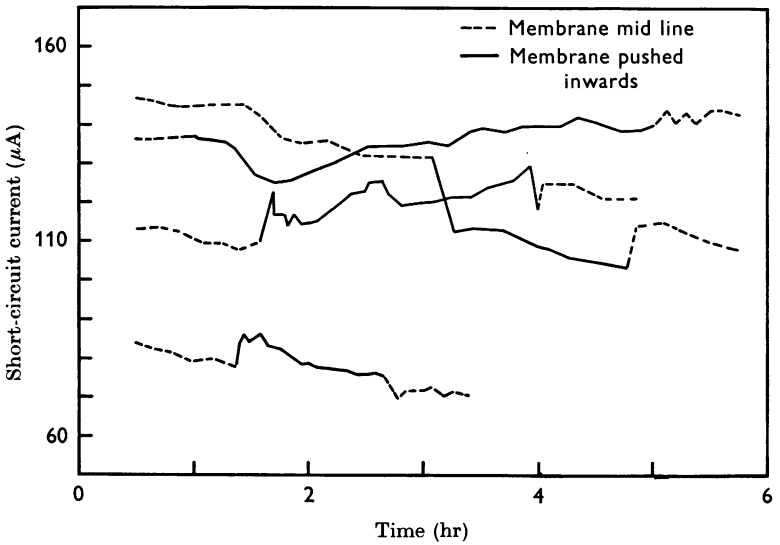

Fig. 5. The effect on the short-circuit current (μA) of bulging isolated living frog-skin membranes in the absence of hydrostatic pressure gradients. The membranes were bulged *inwards* by pushing them with *E* (Fig. 2). Interrupted lines show μA with the membranes in the mid line, and continuous lines show μA with them bulged so that the centre of the membrane was displaced 0·25 in. (6·35 mm), and the surface area increased by 25%.

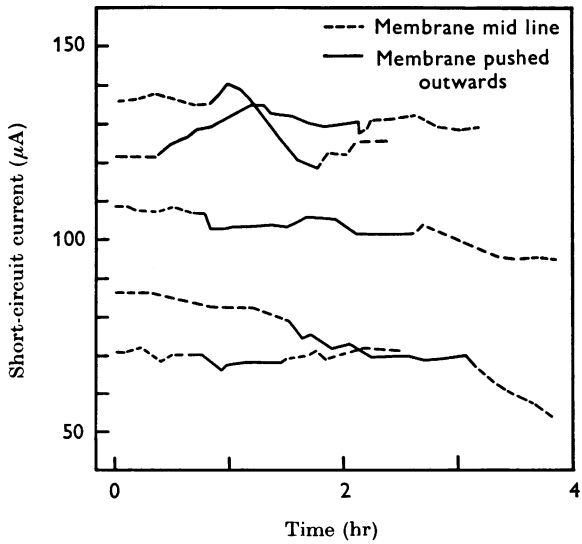

Fig. 6. The effect on the short-circuit current (μA) of bulging isolated living frog-skin membranes in the absence of hydrostatic pressure gradients. The membranes were bulged *outwards* by pushing them with *E* (Fig. 2). Interrupted lines show μA with the membranes in the mid line, and continuous lines show μA with them bulged so that the centre of the membrane was displaced 0·25 in. (6·35 mm) and the surface area increased by 25%.

The effect of bulging frog-skins inwards and outwards by mechanical means in the absence of hydrostatic pressure gradients (Figs. 5 and 6)

Twenty-four skins were studied and in none of them was there any significant change in the short-circuit current when the membrane was bulged in either direction.

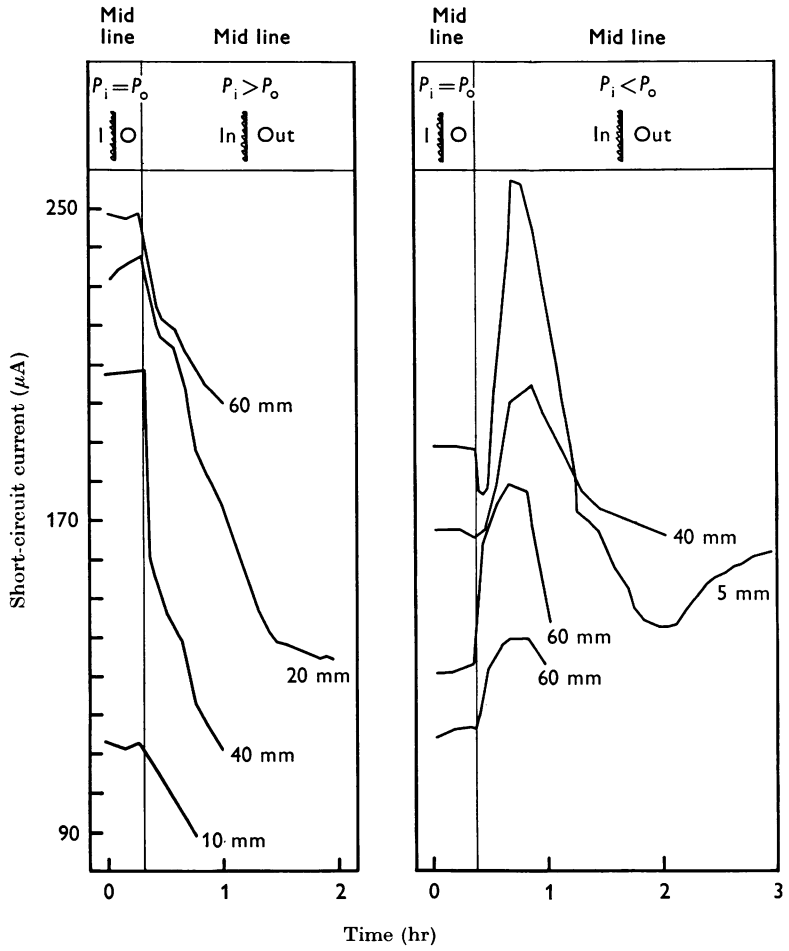

Fig. 7. The effect on short-circuit current (μA) of small constant hydrostatic pressure gradients when the membranes were prevented from bulging by grids placed on each side of them. The figures on the right of each line show the applied pressure gradients in mm H_2O. P_i and P_o are pressures inside and outside the skins respectively.

The effect of applying hydrostatic pressure gradients across frog-skins while they were prevented from bulging by Perspex grids (Fig. 7)

Sixteen skins were studied and in all of them the changes in short-circuit current were similar to those which occurred when skins were allowed to bulge under the influence of the pressure gradients (Fig. 4); when the pressure was higher on the inside of the skin there was a fall in short-circuit current, and when the pressure was higher on the outside of the skin there was an initial rise in current. The most marked response occurred in the skin in which there was a gradient of only 5 mm H_2O.

A comparison of the effect of bulging a skin to the same extent in two ways: (1) by a constant hydrostatic pressure gradient and (2) by mechanical means in the absence of a hydrostatic pressure gradient (Fig. 8)

In three experiments the membrane was bulged inwards mechanically in the absence of a hydrostatic pressure gradient and the short-circuit current did not change significantly from the control value. When the same degree of bulge was taken over by a hydrostatic pressure gradient and the mechanical device was removed, the current rose steadily for 2–4 hr, finally stabilizing at a value above that in the control period. Later when the mechanical device was reintroduced to hold the position of the bulge unchanged and the pressures were equalized, the current at once began to fall and continued to fall for about 2 hr when a stable value was attained. Finally, when the membrane was allowed to return to the mid line by removing the mechanical device, there was no marked change in current.

In three other experiments in which the membrane was bulged towards the outside of the skin there was no change in the current when the skin was bulged mechanically in the absence of a pressure gradient, but there was a marked fall in the short-circuit current when the pressure gradient was introduced to take over the identical bulge. The current fell for about 2 hr and then reached a new steady low level. When the bulge was again taken over mechanically and the pressures were equalized, the current at once rose and continued to rise for nearly 2 hr when it levelled at a value only slight lower than that when the bulge had been caused mechanically at the start of the experiment.

DISCUSSION

The results show that when isolated living frog-skins are bulged by constant hydrostatic pressure gradients of only a few mm H_2O, the short-circuit current shows marked and sustained changes. After any alteration

in pressure gradient the current does not reach a new stable level for 1–4 hr and the higher the initial current, the greater the effect of the gradient. When the bulge is towards the outside of the skin, i.e. when the pressure gradient is in the opposite direction to that of the metabolic pump, the current falls and after an hour or more it reaches a new steady low level; when the bulge is towards the inside of the skin, i.e. when the pressure gradient is in the same direction as that of the metabolic pump, the current

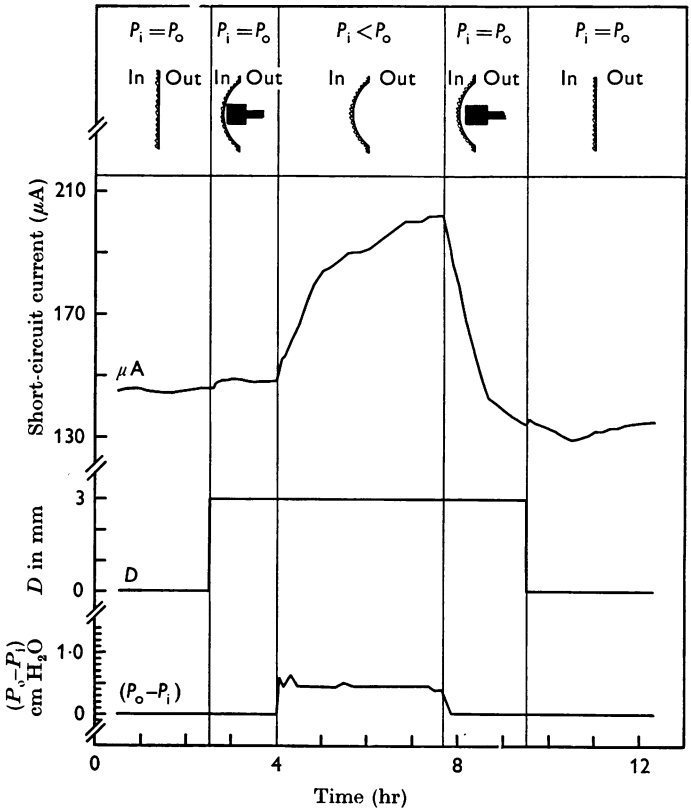

Fig. 8. A comparison of the effect on the short-circuit current (μA) of bulging a skin inwards in two ways: first, by pushing it mechanically in the absence of a pressure gradient, and secondly by means of a constant hydrostatic pressure gradient. P_o and P_i are the pressures in mm H_2O outside and inside the membrane respectively; D is the displacement in mm of the centre of the membrane from the mid line.

rises and is above control values for at least an hour. Similar changes take place when pressure gradients are applied across membranes which are prevented from bulging by Perspex grids. Bulging the membrane mechanically in the absence of a hydrostatic pressure gradient and changing the

hydrostatic pressures equally and simultaneously on both sides of the membrane have no effect on short-circuit current.

Ussing & Zerahn (1951) have shown that in a frog-skin not subjected to a hydrostatic pressure gradient, the electrical current flowing at zero potential difference across the skin is entirely due to active transport of sodium. Although it was not possible, using the methods described above, to prove that this is true when a pressure gradient is present, it seems reasonable to assume that there is a linear relationship between the short-circuit current and the rate of active sodium transport under these conditions too, and that, therefore, the rate of sodium transport rises when the hydrostatic pressure is higher on the outside of the skin and falls when the pressure is higher on the inside.

It is unlikely that the effects of small hydrostatic pressure gradients are due to the changes in surface area, the stretching of the skin or the displacement of cells which accompany bulging, because all these factors are present when the skin is bulged mechanically and then sodium transport is not affected. In addition, when the skin is restrained by Perspex grids, hydrostatic pressure gradients produce changes in sodium transport similar to those obtained when the skin is free to bulge. These grids do not entirely prevent membrane movement, but they restrict it and as the skins are not allowed to bulge through the holes in the grids, the surface area and the tension of the skin are changed much less than when the skin is allowed to bulge. Thus, it would seem that gross changes in the conformation of the skin are not responsible for the effect. However, it is possible that small pressure gradients alter the internal structure of the skin so as to change sodium transport.

Voûte (1963) and Farquhar & Palade (1963, 1964, 1966) have shown by electron microscopy that cells of the frog-skin epidermis are each surrounded by intercellular spaces, continuous with each other, and bridged by desmosomes holding the cells together. These spaces open freely on the inner, dermal side of the epidermis, but appear to be blocked between the cells of the outer layer of the epidermis. Farquhar & Palade (1964) have shown that adenosine triphosphatase activity is localized round the periphery of the epidermal cells, adjacent to the intercellular spaces, and have suggested that this is probably the site of the sodium pump. It is suggested that sodium diffuses passively into the cells from the outer surface of the skin and is then pumped actively into the intercellular spaces. It is probable that, as in the gall-bladder (Diamond & Tormey, 1966), water passively follows the sodium into the spaces, and also that the cells nearest the outside of the skin are the most active in pumping sodium into the spaces (H. H. Ussing & C. L. Voûte, personal communication). As the spaces are open on the dermal side but are at least partially closed on the

outside of the skin, sodium and water tend to flow passively through the spaces down the concentration gradient and also down the internal pressure gradient towards the inside of the skin.

Hydrostatic pressure gradients applied across the skin might affect the rate of sodium transport either by changing the rate of passive diffusion of sodium into the cells on the outside of the skin, or by changing the rate at which the metabolic pumps move sodium from the cells into the intercellular spaces, or by changing the rate at which sodium and water pass down the spaces to the inside of the skin. It is unlikely that the effect of small hydrostatic pressure gradients on sodium transport is due to changes in the rate of passive diffusion of sodium into the cells because it has been shown that the change in sodium transport is greater with small gradients than with large; in addition, it is unlikely that such small gradients would have a marked effect on the rate of diffusion of sodium through the cell pores. On the other hand, it is possible that such gradients may change the rate of flow of sodium and water down the spaces, either directly or indirectly by changing the shape of the exits of the spaces on the inside of the skin. Thus, a rise of pressure on the inside of the skin could directly slow down the bulk-flow and a fall in pressure on the inside of the skin could accelerate it; or the exits to the spaces could be narrowed when the pressure is higher on the inside of the skin, thereby causing a retention of sodium in the channels until the pressure inside the channels rises sufficiently to allow sodium to escape past this obstruction, and conversely, the exits could be wider when the pressure is higher outside the skin, allowing an increased escape of sodium. Such mechanisms involving a change in bulk-flow rate down the spaces would explain the complex nature of the responses and also the length of time taken by the short-circuit current to reach equilibrium, because presumably it would take some time for the whole inter-communicating system of spaces to reach a new steady pressure state following any change in the resistance to the flow. It is also possible that changes in the flow of fluid down the spaces might influence the activity of the sodium pump itself. Thus, when flow is diminished, sodium concentration might rise in the spaces near the pumps and this might inhibit them. Similarly, when the sodium passes rapidly down the spaces, sodium concentration might fall near the pumps and this might stimulate them.

It is possible that these results obtained with isolated frog-skins may be relevant to sodium transport in the proximal tubule of mammalian kidney. The comparison may be valid because there are many similarities in the metabolic nature of the sodium pump in the two membranes and, also, Tisher, Bulger & Trump (1966) have shown that the proximal tubular cells are each surrounded by intercellular spaces, the openings of which are

open at the peritubular border but are blocked on the luminal side. The *outside* of the frog-skin corresponds to the *lumen* of the proximal tubule. If, therefore, it is permissible to apply the results found in the frog-skin to the proximal tubule, a rise in intratubular pressure of a few mm H_2O would increase sodium reabsorption and a rise in peritubular pressure would decrease sodium reabsorption. This is consistent with the results obtained by Gertz (1963), Rector *et al.* (1966), and Brunner *et al.* (1966) who showed that the rate of sodium reabsorption from the proximal tubule is proportional to the tubular volume. Since tubular dilatation and constriction (and hence changes in tubular volume) are brought about by changes in pressure gradient across the tubular wall, and as evidence obtained from the frog-skin indicates that bulging in the absence of hydrostatic pressure gradients has no effect on sodium transport, therefore it seems probable that it is the change in hydrostatic pressure gradient rather than the change in tubular volume which alters sodium reabsorption in the proximal tubule.

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